ATTACHMENT B

Clean Replacement Paragraphs

At the following locations, replace the previously provided paragraph with the following clean paragraph(s).

Page 1, lines 17-19:

In the context of this specification an infertility condition is to be understood to relate not only the capacity to conceive but also to miscarriage, spontaneous abortion or other pregnancy-related conditions, such as pre-eclampsia, and includes sub-fertility.

Page 4, lines 14-29:

The temporal changes in trafficking and phenotypic behavior of endometrial leukocytes during the period between mating and implantation are likely to be orchestrated principally by cytokines emanating from steroid hormone regulated epithelial cells lining the endometrial surface and comprising the endometrial glands (8). Of particular importance are granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-(IL)-6, the synthesis of which is upregulated at least 20-fold and 200-fold respectively in estrogen primed epithelial cells following induction by specific proteinaceous factors in seminal plasma (8.9) known to be derived from the seminal vesicle gland (10). Previous studies have implicated the surge in epithelial GM-CSF release as a key mediator in the post-mating inflammatory response since injection of recombinant GM-CSF into the estrous uterus is sufficient to produce cellular changes resembling those seen following natural mating (11). The inventors have found, using GM-CSF deficient mice, that the chemotactic activity of GM-CSF is likely to be

compensated or augmented by an array of chemokines, the expression of which is transiently upregulated after mating (12), and cytokines synthesised by activated endometrial macrophages including IL-1 and tumour necrosis factor-α (TNF-α)(4).

Page 21, lines 19-30:

Seminal vesicle fluid was fractionated by size exclusion chromatography in order to identify GM-CSF-stimulating activity. Two fractions were identified; a high molecular weight (650 kDA) proteinacous moiety and a intermediate molecular weight, more heterogenous moiety eluting between 150-440 kDA (10.62). The latter moiety was identified as $TGF\beta_1$, on the basis of findings that its GM-CSF stimulating activity was enhanced by acid activation, that $TGF\beta_1$, immunoactivity and bioactivity co-eluted in the same fraction, and that anti-$TGF\beta_1$ neutralizing antibody could block the GM-CSF stimulating activity of this fraction (Figures 1,2). The molecular weight of GM-CSF stimulating activity in seminal vesicle fluid (150-440 kDa) is consistent with that of the latent form of $TGF\beta_1$, a complex of 230-290 kDa which comprises the mature TGF-β dimer (25 kDa) non-covalently associated with a 75-80 kDa latency associated protein and a 130-190 kDa binding protein (23).

Page 23, lines 20-26:

To test the importance of exposure to seminal vesicle fluid for pregnancy success, Balb/c F1 females were mated with CBA males from which the seminal vesicles had been surgically removed (SV-studs). No implantation sites were present in the uterus on day 17 of pregnancy (n=12 females). This total infertility was not due to a lack of

clean paragraphs

fertilization, but rather was associated with implantation failure or early fetal resorption. This may reflect insufficient maternal tolerance of the semi-allogeneic embryos due to the lack or exposure to seminal vesicle fluid TGFβ at mating.

Page 27, line 30 through Page 28, line 4:

Induction of Th 1 hypo-responsiveness against paternal antigens has been reported to result in an improved pregnancy outcome in women previously experiencing recurrent miscarriage. While no data exist on the ability of paternal antigen/TGFβ immunisation to initiate Th 1 hypo-responsiveness against paternal antigens, or to deviate previously existing Th 1 immune responses in women, nor on the ability of TGFβ to improve reproductive outcome, this is likely to be the case. The inventors have been the first to conduct a large randomized, controlled trial investigating the effect of semen exposure on IVF treatment outcome. This trial has confirmed that women exposed to semen (containing paternal antigen and natural TGFβ) around the time of thawed embryo transfer have a reduced risk of early embryonic loss compared to those instructed to abstain (Table VI). This improvement in reproductive outcome is likely to be mediated by maternal immune tolerance towards paternal antigens initiated by TGFβ and seminal antigens at the time of intercourse.

Page 29, lines 18-22:

Pregnancy outcome following thawed embryo transfer. Patient characteristics were not significantly different between the two groups. A biochemical pregnancy was defined as one serum βHCG exceeding 25 IU and a clinical pregnancy as a conceptus/fetal pole

clean paragraphs

seen at ultrasound at 6 weeks gestation. Statistical analysis was performed using the Chi square calculation. NS=not significant. *=one twin pregnancy.

Page 29, lines 33-34:

6. Clark (1984) in Immunological aspects of reproduction in mammals, ed. Crighton, (Butterworths, London), pp. 153-182.

Page 31, line 26:

70. Medawar PB (1953) *Symp Soc Exp Biol* **44**, 320-38.

clean paragraphs